Original Article

Genotoxicity and mutagenicity assessment of electronic cigarette liquids

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Submission: 15-03-2024
Revised: 01-04-2024
Accepted: 03-04-2024
Published: 04-07-2024

Access this article online

Quick Response Code:

Website: https://journals.lww.com/aotm

DOI: 10.4103/atm.atm_59_24

Abstract:

INTRODUCTION: Electronic cigarettes (e-cigarettes) are often advertised as a safer alternative to traditional smoking. However, recent data suggest they may not be as safe as previously believed. This study aims to evaluate the genotoxicity and mutagenicity of e-cigarette liquids.

METHODS: We randomly selected eight varieties of e-cigarette liquids from the local market in Jeddah, Saudi Arabia. We evaluated their genotoxicity using the Genotoxicity SOS-Chromo Test™ Kit. In this investigation, a rat liver S9 fraction was utilized to emulate liver metabolic function to measure any chemical substance's mutagenic potential. The SOS-Chromo Test was performed by recording the β-galactosidase and alkaline phosphatase activity with and without the metabolic activation enzyme (S-9).

RESULTS: All samples, except for the first two dilutions of sample 2, were nongenotoxic in the absence of the S9 activation enzyme, according to the genotoxicity analysis. However, when tested in the presence of the S9 enzyme, samples 2, 4, and 7 exhibited mutagenic activity at varying concentrations.

CONCLUSION: Contrary to common belief, e-cigarettes are not safe. The present investigation confirms the presence of both toxicants and carcinogens in some e-cigarette liquids. This exposure could increase users' risk of various health complications.

Keywords:

Electronic cigarette, genotoxicity, mutagenicity, smoking, vaping

The use of electronic cigarettes (e-cigarettes) is rapidly increasing worldwide.[1] These devices have gained popularity primarily among habitual smokers.[2] They are promoted as a safer alternative to traditional smoking.[3] E-cigarettes, as defined by the Food and Drug Administration (FDA), are battery-operated devices that deliver nicotine and other chemicals. They work by heating a liquid solution, termed "juice," which then produces a vapor. This vapor is inhaled, providing the smoker with a nicotine dose. Since vapor production does not involve combustion, manufacturers assert it lacks the chemicals and carcinogens typically produced by traditional cigarette combustion, making it a safer choice.[4] Moreover, many constituents of e-cigarette liquids are included in the FDA's "Generally Recognized As Safe" list.

While e-cigarette liquids do not contain tobacco, they may contain tobacco-related compounds like nitrosamines.[5] Past research has found various toxic substances in the vapor produced, including carbonyl products, heavy metals, and other toxins.[6-8] In addition, there is evidence suggesting that e-cigarette smoking can potentially harm the human cellular system.[9]

Data on the genotoxicity and mutagenicity of compounds in e-cigarette cartridges and nicotine refill solutions are limited.[10] Some

How to cite this article: Al-Otaibi HM, Baqasi AMS, Alhadrami HA. Genotoxicity and mutagenicity assessment of electronic cigarette liquids. Ann Thorac Med 2024;19:222-7.

investigations have found little to no potentially harmful substances,[8] while others have discovered significant cellular damage and functional alterations.[11,12] Thus, there is an urgent need to create and adopt sensitive, accurate, simple, and cost-effective techniques for screening and identifying harmful properties such as genotoxicity and mutagenicity that may be present in numerous e-cigarette juices.

Biosensors used for genotoxicity screening have consistently shown alignment with traditional bioassays and offer several advantages, requiring only minor laboratory equipment and space requirements.[13] The term "biosensor" refers to an array of biological materials that yield a measurable signal through an appropriate transducer when exposed to genotoxic and carcinogenic substances.[13] The ubiquity of genotoxic substances has necessitated the creation of biosensors designed to screen a vast number of samples for damaging properties such as genotoxicity and carcinogenicity.[13,14]

In this study, the SOS-Chromo test was employed to assess the genotoxicity of e-cigarette liquids. The SOS-Chromo test is based on the naturally occurring SOS DNA damage and repair tolerance system in *E. coli* PQ37.[15,16] This system responds to the presence of a broad spectrum of genotoxic materials by gauging the reporter gene β-galactosidase (β-gal).[17] Regarded as a straightforward, fast, and adaptable test, the colorimetric SOS-Chromo test assay can estimate the genotoxicity of various substances in numerous situations.[18] *E. coli* PQ37 is genetically engineered through the fusion of the *sfiA* gene – a component of the SOS system – with the *lacZ* gene, which facilitates the synthesis of β-gal.[19] The SOS-Chromo Test showcases several beneficial attributes, such as accuracy and sensitivity, in addition to being less time-consuming compared to the Ames test.[20]

The main goal of this study is to examine the genotoxicity and mutagenicity of randomly selected e-cigarette liquids, sold in Saudi Arabia's local market.

Methods

Toxicity and mutagenicity assessment using the SOS-Chromo Test

Eight distinct e-cigarette liquid types were randomly chosen from the local market in Jeddah, Saudi Arabia. The nicotine concentrations in these various e-cigarette liquids are depicted in Table 1.

The genotoxicity of eight assorted e-cigarette liquids was assessed using an analytical Genotoxicity SOS-Chromo Test™ Kit from EBPI (Environmental

Table 1: Nicotine concentrations in different electronic cigarette liquids

E-cig liquid number	Nicotine concentrations (mg/mL)	E-cig liquid number	Nicotine concentrations (mg/mL)
E-cig 1	2	E-cig 2	10
E-cig 3	3	E-cig 4	6
E-cig 5	4	E-cig 6	1
E-cig 7	5	E-cig 8	0.5

E-cig, Electronic cigarette

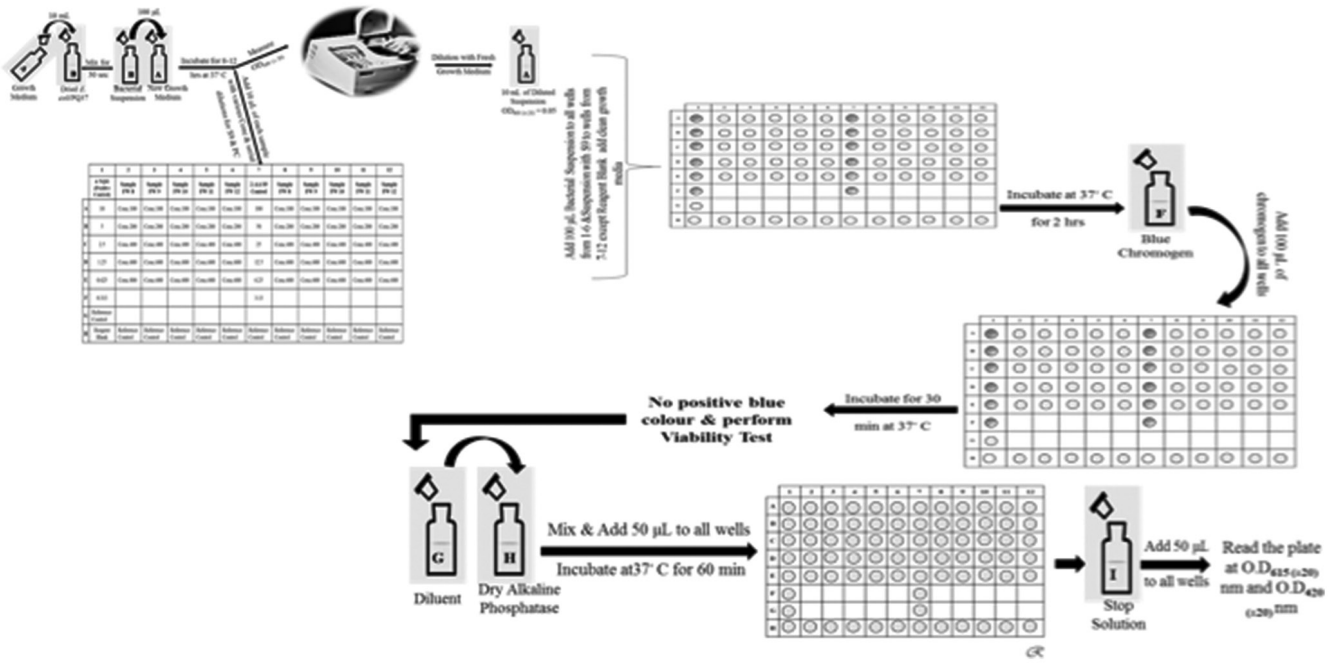

Figure 1: Design of genotoxicity/mutagenicity experimental steps

Bio-Detection Products Inc., Mississauga, Ontario, Canada). The mutagenic potential of various chemicals was measured by simulating rat liver S9 fraction as a stand-in for liver function metabolism. Activities of β-gal and alkaline phosphatase (AP) were evaluated in the SOS-Chromo Test in both the presence and absence of a metabolic activation enzyme (S-9). The procedure for the entire SOS-Chromo Test is outlined in Figure 1.

Resuscitation of lyophilized bacteria

The lyophilized bacteria were revived by adding 10 mL of growth media. After 30 s, 100 µL of this bacterial suspension was gently transferred to a fresh growth medium. The mixture was then incubated overnight for 8–12 h in a rotary shaker set to 150 rpm and a temperature of 37°C.

The overnight bacterial culture was diluted to a final OD of 0.05 at 600 nm using a fresh growth medium. We used the following equation (equation 1) to calculate the required volume for dilution:

$$\text{The required volume of culture} = \frac{0.5}{\text{OD of overnight culture}}$$

Equation 1: Calculating the volume of bacterial suspension

SOS-Chromo Test experiment

The experiment was performed using two microtiter plates. Every well contained 10 µL aliquot of 10% dimethyl sulfoxide, excluding the first row (A). In the first plate, 20 µL of tested samples and the positive control 4-Nitroquinoline-N-Oxide (4-NQO) were dispensed into their respective wells in the first row. Then, 100 µL of a diluted bacterial suspension was added into each well after undergoing serial dilutions, with the exception of row H, assigned as the reference control. The same process was duplicated on the second plate, but 4-NQO was replaced with the S9 positive control, 2-Aminoanthracene. Both plates, after mixing the bacterial suspension with the S9 mixture, were incubated for 120 min at 37°C. The optical density of the plates was measured at ($OD_{615} \pm_{20}$) to detect genotoxic activity and at ($OD_{420} \pm_{20}$) to monitor cell viability.

Results

Mutagenicity assessment

The tests for genotoxicity and mutagenicity were performed by measuring the beta-gal enzyme activity, an indication of DNA damage. Furthermore, cell viability was monitored through AP expression when exposed to e-cigarette solution samples. The genotoxicity/mutagenicity results were then analyzed by determining the SOS-inducing potency (SOSIP) for each sample at every dilution using equation 2.

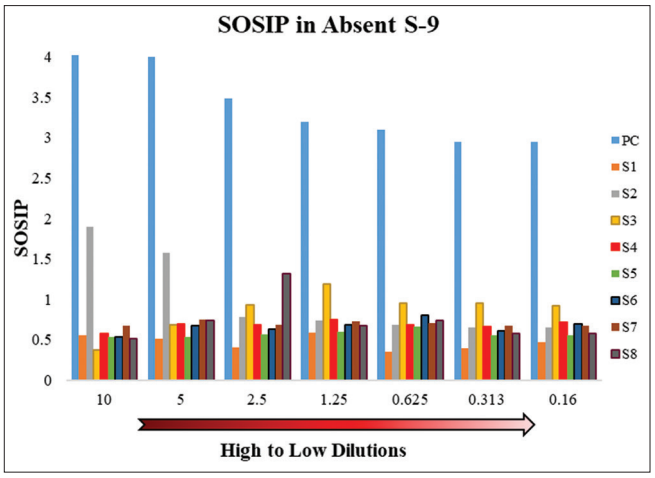

Figure 2: Genotoxicity assessment of different concentrations of the electronic cigarette samples

Table 2: Genotoxicity/mutagenicity assessment of different concentrations of the electronic cigarette samples

Sample 1	Value	Sample 2	Value	Sample 3	Value
1st dilution	0.56	1st dilution	1.9	1st dilution	0.37
2nd dilution	0.51	2nd dilution	1.58	2nd dilution	0.68
3rd dilution	0.41	3rd dilution	0.78	3rd dilution	0.93
4th dilution	0.59	4th dilution	0.74	4th dilution	1.19
5th dilution	0.35	5th dilution	0.69	5th dilution	0.95
6th dilution	0.40	6th dilution	0.65	6th dilution	0.95
7th dilution	0.47	7th dilution	0.65	7th dilution	0.92
Sample 4	**Value**	**Sample 5**	**Value**	**Sample 6**	**Value**
1st dilution	0.58	1st dilution	0.53	1st dilution	0.53
2nd dilution	0.70	2nd dilution	0.53	2nd dilution	0.67
3rd dilution	0.69	3rd dilution	0.57	3rd dilution	0.63
4th dilution	0.75	4th dilution	0.60	4th dilution	0.68
5th dilution	0.69	5th dilution	0.66	5th dilution	0.80
6th dilution	0.66	6th dilution	0.56	6th dilution	0.61
7th dilution	0.72	7th dilution	0.56	7th dilution	0.70
Sample 7	**Value**	**Sample 8**	**Value**		
1st dilution	0.67	1st dilution	0.51		
2nd dilution	0.75	2nd dilution	0.74		
3rd dilution	0.69	3rd dilution	1.32		
4th dilution	0.73	4th dilution	0.67		
5th dilution	0.71	5th dilution	0.74		
6th dilution	0.67	6th dilution	0.58		
7th dilution	0.67	7th dilution	0.58		

Yellow indicates genotoxicity, red indicates inconclusive results, and colorless indicates nongenotoxicity

$$\text{SOIP} = \frac{(\text{O.D}_{630}i) \div (\text{O.D}_{405}i)}{(\text{O.D}_{630}\text{NC}) \div (\text{O.D}_{405}\text{NC})}$$

Equation 2: Calculation of SOS-inducing potency.

The results, categorized by the SOSIP classification, were calculated both with and without the presence of the S9 activation enzyme. Genotoxicity analysis found all samples tested without S9 to be nongenotoxic, with the exception

Table 3: SOS-Chromo Test values for the second plate with S9 activation enzyme after performing the SOS-inducing potency equation

Sample 1	Value	Sample 2	Value	Sample 3	Value
1st dilution	1.4	1st dilution	8.16	1st dilution	1.12
2nd dilution	0.86	2nd dilution	6.35	2nd dilution	0.96
3rd dilution	0.88	3rd dilution	2.95	3rd dilution	0.80
4th dilution	0.75	4th dilution	2.87	4th dilution	1.04
5th dilution	0.67	5th dilution	2.27	5th dilution	0.94
6th dilution	0.75	6th dilution	2.56	6th dilution	0.67
7th dilution	1.3	7th dilution	2.68	7th dilution	0.79
Sample 4	**Value**	**Sample 5**	**Value**	**Sample 6**	**Value**
1st dilution	2.12	1st dilution	0.97	1st dilution	1.11
2nd dilution	2.15	2nd dilution	1.01	2nd dilution	0.98
3rd dilution	2.44	3rd dilution	1.12	3rd dilution	1.32
4th dilution	1.98	4th dilution	1.45	4th dilution	1.11
5th dilution	2.89	5th dilution	1.05	5th dilution	0.87
6th dilution	2.10	6th dilution	1.13	6th dilution	1.17
7th dilution	2.18	7th dilution	0.73	7th dilution	1.04
Sample 7	**Value**	**Sample 8**	**Value**		
1st dilution	0.72	1st dilution	0.67		
2nd dilution	0.79	2nd dilution	1.26		
3rd dilution	0.76	3rd dilution	0.64		
4th dilution	0.67	4th dilution	0.67		
5th dilution	0.78	5th dilution	0.71		
6th dilution	0.58	6th dilution	0.77		
7th dilution	1.93	7th dilution	0.48		

Yellow indicates genotoxicity, red indicates inconclusive results, and colorless indicates nongenotoxicity

of the initial two dilutions of sample 2, which showed genotoxicity. Table 2 contains the genotoxicity analysis results for all samples subjected to testing without S9.

The analysis of the SOSIP results, conducted in the presence of the S9 activation enzyme, demonstrated the capability of e-cigarette samples to induce mutagenicity. All tested samples exhibited nonmutagenic activities at various concentrations, with the exception of samples 2, 4, and 7 in the presence of the S9 activation enzyme. Table 3 provides the SOS-Chromo Test values for the second plate, having implemented the SOSIP equation and included the S9 activation enzyme.

The SOSIP results showed a mutagenic effect for samples 2 and 4 at all dilutions, with ambiguous results at the 12.5% (1:8) dilution of sample 4. Conversely, sample 7 yielded nonmutagenic results, with the exception of an inconclusive result at the 1.6% (1:64) dilution. Figure 2 presents a summary of the SOSIP analysis, demonstrating the absence and presence of S9 metabolic activation enzyme in all e-cigarette sample concentrations.

Discussion

The current study reveals that in the absence of the S9 enzyme, all samples tested were nongenotoxic, with the exception of the first two dilutions of sample 2, which exhibited genotoxicity. Mutagenic activity was observed in three samples (samples 2, 4, and 7) tested at varying concentrations with the S9 activation enzyme. These results indicate that e-cigarette liquids, in their standard form, may not be as harmless as previously assumed. Genotoxicity and mutagenicity are evident in certain types of e-cigarette liquids available on the local market.

Biosensors for genotoxicity assessment are compelling due to their consistency with traditional bioassays and their capability to detect various genotoxic contaminants with minimal laboratory equipment and space requirements.[14] Although there is a debate concerning the predictability of genotoxicity and carcinogenicity through microbial biosensors, most studies report a strong correlation between the carcinogenic effects of compounds on microbial biosensors and their genotoxic and tumor-induced properties in mammals.[13] Due to their simplicity, specificity, and sensitivity, microbial biosensors are ideal tools for detecting and screening genotoxic and carcinogenic substances in pharmaceutical and medical research.[14] These have been employed in research laboratories and regulatory agencies worldwide for over two decades.[14]

E-cigarette liquids typically consist of propylene glycol and vegetable glycerin mixed at various ratios and can include nicotine at different concentrations. Over 7000 e-cigarette liquid flavors are commercially available.[21] Most of these ingredients are labeled safe for ingestion as long as they are below their toxic concentration. These liquids can also contain natural extracts such as herbal and tobacco extracts, as well as essential oils. The composition of these extracts can vary based on their biological and geographical origins.[22] For instance, pulegone, an organic compound found in the oil from mint plants, was detected in e-cigarette liquid at a level that exceeds its toxic threshold.[23] This compound has been reported as carcinogenic.[24] Hence, e-cigarette liquids may contain unknown substances with unidentified toxicological properties.[25] These substances might pose a health risk, especially if their concentration surpasses their toxic levels. It has been reported that the concentration of some flavorings exceeded the levels necessary to induce genotoxicity.[10] Data from the American Association of Poison Control Centers in 2015 indicate approximately 3000 cases of exposure to e-cigarette liquid, with almost a third of these cases needing medical attention.[26] Ingesting e-cigarette liquid can prove fatal, with several deaths in both children and adults reported following ingestion.[27,28] Therefore, current data reinforce the fact that e-cigarette liquids are not harm-free, and some of the commercially available ones contain toxic substances.

Previous data suggest that various types of e-cigarette liquids have differing levels of genotoxicity and mutagenicity. Studies like that of Al-Saleh *et al*. have confirmed these e-cigarette liquids' ability to cause DNA damage, break chromosomes, and kill cells.[29] Others have identified cytotoxic impacts on oropharyngeal mucosal tissues.[30]

Recent research has evaluated the toxic effects of a combination of flavoring chemicals, propylene glycol, and vegetable glycerin on liver cells, finding that these flavoring chemicals can reduce the cells' viability.[31] Behar *et al*., in 2014,[12] analyzed eight cinnamon-flavored e-cigarette liquids for toxicity, revealing variations in cytotoxicity levels, with most being cytotoxic.[12] It was noted that the cytotoxicity of e-cigarette liquid primarily results from the concentration and number of flavors, not the nicotine content.[32]

Furthermore, studies have shown that e-cigarette liquids and their resulting aerosols are cytotoxic, with the aerosol's cytotoxicity being predictable from the liquid 74% of the time.[33] Behar *et al*. reported that over 20 of the 39 e-cigarette liquids they tested contained compounds toxic to lung cells.[34] This study aims to evaluate the genotoxicity of e-cigarette liquids commercially available. An in-depth analysis of each e-cigarette liquid component's genotoxicity is not part of this research. However, the genotoxicity of various e-cigarette liquids is evident from existing data.

The current study reveals the presence of toxic and carcinogenic elements in e-cigarette liquids. E-cigarette manufacturers often argue that toxicity and carcinogens stem primarily from tobacco combustion. As e-cigarettes use a heating system to aerosolize liquid instead of combustion, manufacturers claim they present a safer alternative to conventional smoking. However, this assertion is scientifically unfounded.[35] Increasing evidence now indicates that e-cigarettes are harmful in both their liquid and vapor forms.[36] The e-cigarette liquid contains harmful and carcinogenic substances. Classifying other e-cigarette liquid components as safe does not negate their genotoxicity when aerosolized.[37] Therefore, the toxic effects of e-cigarette products could be attributed to one of two mechanisms. These may either arise from the inherent toxicity of the base elements used to formulate the e-cigarette liquids or due to the transformation or formulation of new compounds when the solution is heated or reacts with the heating coil.[38]

This study is not without its limitations. Primarily, our data reveal the presence of various toxicants in different types of e-cigarette liquids, yet we did not identify the specific compounds. The study aimed to assess the genotoxicity of e-cigarette liquids in their original form, aiding in the confirmation or debunking of the assumption that e-cigarette smoking is harmless. Second, our team did not investigate the genotoxicity of e-cigarette liquids in their vapor state. The main objective of this research was to evaluate the genotoxicity of unregulated, commercially available e-cigarette liquids. Previous reports have suggested that the toxicity of e-cigarette liquids anticipates the toxicity of the aerosols 74% of the time.[33] However, assessing the genotoxicity of aerosolized e-cigarette liquid would provide significant insights into the data presented.

Conclusion

E-cigarettes are not as safe as they may seem. Evidence is increasingly suggesting that they are not risk-free. They have been found to contain toxic and carcinogenic substances in both their liquid and vapor forms. Further research is required to identify more of these harmful compounds.

Financial support and sponsorship

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant no. G: 1283-883-1440. The authors, therefore, acknowledge with thanks DSR for technical and financial support.

Conflicts of interest

There are no conflicts of interest.

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